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Slight Instability of a FMR-1 Allele Over Three Generations in a Family From the General Population

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We report on a family segregating a *FMR-1* allele within the "grey zone" of triplet repeat length (n = 51). The allele showed a 1-unit increment when transmitted through a female meiosis and a 1-unit increment when transmitted through a male of the next generation. At the following generation, a pregnant woman had amniocentesis performed. The latter showed she transmitted the allele unchanged (n = 53) to her male fetus.

This family was not ascertained through an affected subject, and there was no family history of mental retardation. Thus our observation reflects the natural history of an unstable allele in the general population. Systematic analysis of such alleles may help refine our understanding of the grey zone of triplet repeat length. © 1996 Wiley-Liss, Inc.

KEY WORDS: fragile X, premutation,triplet repeat, FMR-1, unstable DNA

INTRODUCTION

Since the discovery of triplet repeat expansion in fragile X [Fu et al., 1991], an overlap has been noted in the lengths of normal, stable alleles, and premutated, unstable alleles. This led to the concept of a "grey zone" ranging from approximately 40 to 55 repeats, in which length analysis alone is not sufficient to predict stability upon transmission. Besides length, the interruption of the CGG repeat tract by AGG trinucleotides is a major determinant of stability [Eichler et al., 1994].

Fragile X is a prevalent disorder. The prevalence of the premutation, as defined only by an arbitrary size criteria, might be as high as 1% in some population samples [Snow et al., 1993]. In a cohort of 4,200 unselected consecutive women attending the obstetric-gynecology facilities of hospitals affiliated to our University, we found a frequency of 1/250 carriers of alleles with n ≥ 54 (PC, unpublished results). Because of the prevalence of alleles within the premutation size range, the feasibility of carrier screening in the general population has come to attention. Screening remains controversial however, mainly because of the uncertainty regarding alleles belonging to the grey zone.

We report on a *FMR-1* allele from the general population that showed a 1 unit increment over two generations, and that was stably transmitted from a mother to her male fetus thereafter.

MATERIALS AND METHODS

Total blood or amniocyte DNA was digested by *Eco*RI, *Eco*RI, and *Eag*I, or *Bgl*II and probed with StB12.3 as described [Rousseau et al., 1991]. PCR using primers flanking the CGG repeat tract and ^{32}P radiolabeled dCTP was performed using a protocol developed in our laboratory (primer sequences and detailed protocol available upon request), and run on a 0.4 mm, 6% polyacrylamide 8 M urea denaturing gel. Samples from patients carrying premutations of known sizes were included as positive controls and size standards, along with M13 phage dideoxy sequence reaction products used as molecular size markers.

RESULTS

In a pilot effort to screen carrier women from the general population attending the obstetric-gynecology facilities affiliated to our University Hospital, a 25-year-old Caucasian had blood DNA analysis performed in the 6th week of her first pregnancy for cystic fibrosis and Fragile X carrier detection. There was no family history of mental retardation, development delay, or learning disability. A Southern blot probed with StB12.3 showed a doublet DNA band, and the repeat numbers of her *FMR-1* genes were measured at 53 and 28 by PCR analysis. In an attempt to evaluate the stability of the n = 53 allele, a family analysis was performed (Fig. 1). The allele was not stable. It was inherited via the consultant's father, from the paternal grandmother, in whom it displayed 52 and 51 repeats,

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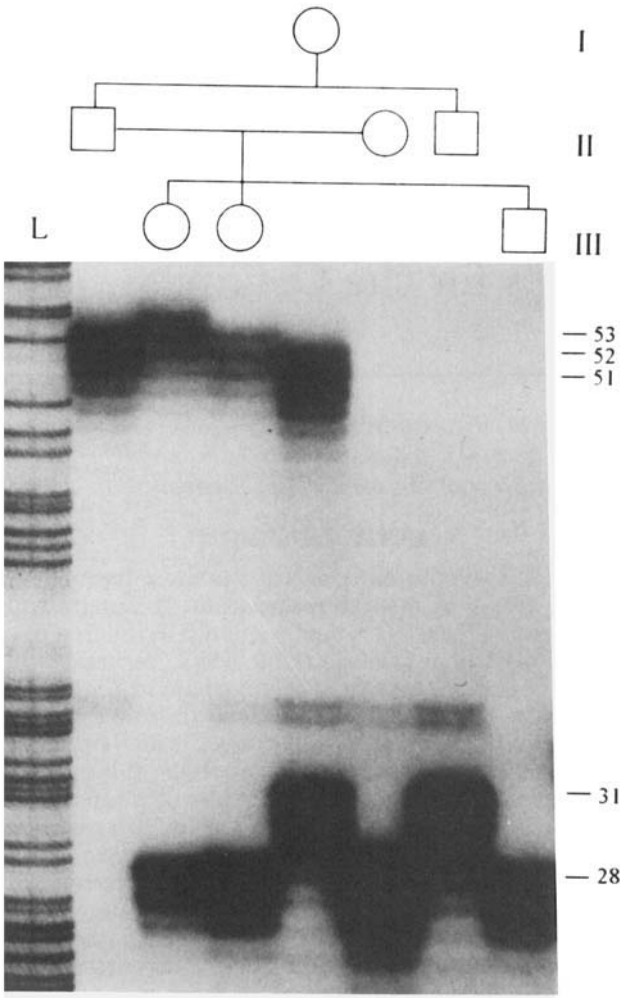

Fig. 1. Family analysis of the number of CGG repeats. The CGG repeat tract of the *FMR-1* gene was amplified by PCR using flanking primers and ^{32}P radiolabeled dCTP. The products were run on a denaturing polyacrylamide gel along with a M13 phage sequence reaction product used as a size marker (L). The generations are labeled in roman numbers. Arabic numbers indicate the number of triplet repeats; the larger allele is measured as 51 in the maternal grandmother, 52 in the consultant's father, 53 in the consultant, and 52 in her sister. The consultant was pregnant, and PCR analysis showed her fetus had inherited the n = 53 allele unchanged (not shown).

respectively. The father (n = 52) transmitted the allele unchanged to one of his two daughters, and transmitted it with a 1 unit increment to the consultant. After discussion of the risks with the prospective parents in a second genetic counseling session, amniocentesis was performed and fetal DNA was analysed. The fetus was found to be a male who had inherited the premutated allele. Its size had remained unchanged (n = 53). A Southern blot analysis using the StB12.3 probe on amniocyte was not contributive. The results were conveyed to the prospective parents on a third session, and the counseling was reassuring. The pregnancy continued uneventfully.

DISCUSSION

We present an unstable *FMR-1* allele in the grey zone that displays a 1 unit increment through both 1 male and 1 female transmissions, and was stably transmitted through a female meiosis while largest in size (53 repeats in the prospective mother and her male fetus).

Apart from triplet repeat expansion, in which polymerase slippage is the favored model [Eichler et al., 1993], other mechanisms may account for changes in tandem repeat number. Unequal sister chromatid exchange and gene conversion are believed to underlie the variability of minisatellite length [Jeffreys et al., 1994]. As we observed two consecutive events of minimal increase in length, it is very unlikely that such mechanisms are involved. A next step of our study should consist of sequencing of the repeat tract in search of AGG interruptions or other point mutations.

Most studies that correlate the numbers of repeats with the risk of expansion to the full mutation are biased for the high risks, as subjects were ascertained through an affected individual. Furthermore, it is still unknown whether all unstable alleles have a propensity to expand to the full mutation state. Systematic analysis of unstable alleles from the general population, such as the one reported here, may help refine the risk figures associated with repeat numbers, especially in the grey zone.

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